

EVALUATION OF ANTIMICROBIAL POTENTIAL OF DIFFERENT SOLVENT EXTRACTS OF SOME MEDICINAL PLANTS OF SEMI-ARID REGION

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ABSTRACT

Objective: Infections caused by microorganisms that have become resistant to commonly used antibiotics have become a major nuisance globally. The problem of microbial resistance is increasing rapidly, and therefore there is an urgent need to develop novel antimicrobial agents from a natural source. The aim of the present study was to evaluate the antimicrobial activity of different solvent extracts of aerial part of *Alysicarpus procumbens*, *Fimbristylis dichotoma*, *Saccharum spontaneum*, *Suaeda nigra*, and *Typha angustifolia* against pathogenic microorganisms.

Methods: The extraction was done by individual cold percolation method using five solvents of different polarity, viz., petroleum ether, ethyl acetate, acetone, methanol, and water (aqueous). The antimicrobial activity was done by agar well diffusion method against Gram-positive and Gram-negative bacteria and fungi.

Results: All the plant solvent extracts showed varied level of antimicrobial activity against different microorganisms. All extracts of five plants showed better antibacterial activity than antifungal activity; Gram-positive bacteria were more susceptible than Gram-negative bacteria.

Conclusion: The polarity of solvent greatly influences extractive yield and antimicrobial activity of medicinal plants. The best activity was shown by solvent extracts of *S. nigra*. Hence, it can be considered as good source of antimicrobial agents.

Keywords: Medicinal plants, Antimicrobial activity, Extractive yield, Solvent extracts.

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INTRODUCTION

The use of plants as herbal medicine is widespread from ancient time. Even today in the times of advanced technology, medical science use plants for the treatment and curing of various diseases. The medicinal plants are considered as a rich source of bioactive phytochemicals which can be used in drug development [1]. Medicinal plants are frequently used for extraction of active constituents which are used in the preparation of different drugs [2]. Medicinal plants have been tested for a wide range of biological, activity such as antifungal [3], antioxidant [4], anti-inflammatory [5], hepatoprotective [6], antidiabetic [7], and anticancer [8] activities. Nowadays, the incidence of multiple drug resistance in pathogenic microorganisms is increasing largely. The number of multidrug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing [9]. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation, and ongoing epidemics of HIV infection [10,11].

Available synthetic drugs have side effects, adulteration and are also expensive and inadequate for the treatment of diseases. Therefore, there is need to search for a new infection fighting approach to control microbial infections [12]. This has lead to discover novel active compounds against resistance microbes with a novel mode of actions and new targets; this has become an urgent need. An attractive, advantageous and alternative approach is the use of medicinal plants. The plant based medicines are relatively safer than synthetic alternatives, very few side effects, high effectiveness, offering intense therapeutic benefits, and inexpensive treatment.

Plant produces a wide variety of secondary metabolites such as saponins, tannins, steroids, terpenoids, resins, and flavonoids which

are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells, but their concentration varies according to the plant parts, season climate and particular growth phase [13]. Hence, in the present work five plants, viz., *Alysicarpus procumbens*, *Fimbristylis dichotoma*, *Saccharum spontaneum*, *Suaeda nigra*, and *Typha angustifolia* were selected to evaluate its antimicrobial potential against pathogenic microorganisms.

METHODS

Plant collection

Five plants were collected from Jamnagar and Surendranagar districts, Gujarat, India. The plant was compared with voucher specimen deposited at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The aerial parts of five plants, i.e., *A. procumbens* Roxb. (AP) (Voucher Specimen No. PSN143), *F. dichotoma* L. (FD) (Voucher Specimen No. PSN772), *S. spontaneum* L. (SS) (Voucher Specimen No. PSN848), *S. nigra* L. (SN) (Voucher Specimen No. SU/BIO/520/Thakrar), and *T. angustifolia* L. (TA) (Voucher Specimen No. SU/BIO/522/Thakrar). They were thoroughly washed with tap water and dried under shade. The dried aerial parts were homogenized to a fine powder and stored in air tight bottles which were later used for solvent extraction.

Extraction

Individual cold percolation method

The dried powder of aerial parts of five plants was extracted individually by cold percolation method [14] using different organic solvents such as petroleum ether (PE), ethyl acetate (EA), acetone (AC), methanol (ME),

and water (AQ). 10 g of dried powder was added to 100 ml of PE in a conical flask, which was plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 hrs. After 24 hrs, the extract was filtered with eight layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the solvent was evaporated. The residue was then added to 100 ml of solvents (EA, acetone, methanol, and water) in different conical flasks, which were plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 hrs. After 24 hrs, the extract was filtered with eight layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the solvent was evaporated. The dry extract was stored at 4°C in airtight bottles; the extracts were weighed to obtain the extraction yield.

Antimicrobial activity

Antimicrobial activity was done by agar well diffusion method against Gram-positive bacteria, Gram-negative bacteria and fungal strains. The microorganisms were obtained from National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4°C. The bacteria and fungi were maintained on nutrient agar and MGY medium (Hi Media, India), respectively. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Bacillus subtilis* (BS) ATCC6633, *Staphylococcus aureus* (SA) ATCC29737, and *Corynebacterium rubrum* (CR) ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa* (PA) ATCC9027, *Salmonella typhimurium* (ST) ATCC23564, and *Klebsiella pneumonia* (KP) NCIM2719. The fungi studied were *Candida albicans* (CA) ATCC2091, *Cryptococcus neoformans* (CN) ATCC34664, *Candida glabrata* (CG) NCIM3448, and *Candida epicola* (CE) NCIM3367.

Agar well diffusion assay

In vitro antimicrobial activity of different extracts of *A. procumbens*, *F. dichotoma*, *S. spontaneum*, *S. nigra*, and *T. angustifolia* aerial parts were determined by agar well diffusion assay [15,16]. Mueller-Hinton agar and sabouraud dextrose agar were used for bacteria and fungi, respectively. Mueller Hinton agar and sabouraud dextrose agar (40-42°C) were seed with 200 µl of inoculums (1×10^8 cfu/ml) and poured into Petri dishes. The media was allowed to solidify, and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Different solvent extracts were dissolved in 100% dimethyl sulfoxide (DMSO) at a concentration of 20 mg/ml; from this 100 µl of the extract was added into the 8.5 mm diameter well. The plates were incubated at 37°C and 28°C for 24 and 48 hrs for bacteria and fungi, respectively. DMSO was used as a negative control. Antimicrobial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate, and the average values are presented.

RESULTS AND DISCUSSION

Extractive yield

The extractive yield of different solvent extracts of aerial parts of five different plants is given in Fig. 1. The extractive yield was different in different solvent extracts of different plants. In all the five plants, maximum extractive yield was in methanol and aqueous extracts. The extractive yield was maximum in *S. nigra* and minimum in *S. spontaneum*. The extractive yield was minimum in PE, EA, and acetone extracts in all the five plants. The results support the idea that the solvent greatly affects the extractive yield, antimicrobial and antioxidant activity as also reported by Fernandez-Agullo *et al.*, [17], Chanda *et al.*, [18], Al-Farsi and Lee, [19] and Ammar *et al.*, [20].

Antimicrobial activity

Antimicrobial activity of *A. procumbens*

Antimicrobial activity of different solvent extracts of *A. procumbens* against microorganisms is given in Fig. 2. Different solvent extracts showed a different level of activity against the tested microbial strains. All the extracts showed maximum antimicrobial activity toward Gram-positive bacteria as compared to Gram-negative bacteria and fungi. Gram-positive bacteria, *B. cereus* and *B. subtilis* were inhibited by all the five extracts. *S. aureus* was inhibited by semi polar solvent EA and aqueous extract; while *C. rubrum* was inhibited by only semi polar solvent EA and polar solvent acetone extract (Fig. 2a). Gram-negative bacteria, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *K. pneumoniae* were not inhibited by any of the five solvent extracts (Fig. 2b). Fungi, *C. epicola* and *C. glabrata* were the most resistant fungal strains. *C. albicans* was inhibited by semi polar solvent EA and polar solvent acetone extracts. *C. neoformans* was inhibited by polar solvent acetone and methanol extracts and aqueous extracts (Fig. 2c). In general, EA and acetone extracts showed maximum antimicrobial activity. *B. cereus* and *B. subtilis* were the most susceptible bacterial strains.

Antimicrobial activity of *F. dichotoma*

Antimicrobial activity of different solvent extracts of *F. dichotoma* against microorganisms is given in Fig. 3. All the extracts showed moderate antimicrobial activity against microorganisms. The Gram-positive bacteria, *S. aureus* was inhibited by semi polar solvent EA and polar solvent methanol extracts. *B. subtilis* and *C. rubrum* were inhibited by only polar solvent acetone extract. *B. cereus* was the most resistant bacterial strain (Fig. 3a). The Gram-negative bacteria, *P. aeruginosa* were inhibited by all the five extracts. *E. coli* was inhibited by non-polar solvent PE, semi polar solvent EA, polar solvent acetone, and methanol extracts. *S. typhimurium* and *K. pneumoniae* were the most resistant bacterial strains (Fig. 3b). Fungi, *C. epicola* and *C. glabrata* were the most resistant fungal strains. *C. albicans* was inhibited by all the

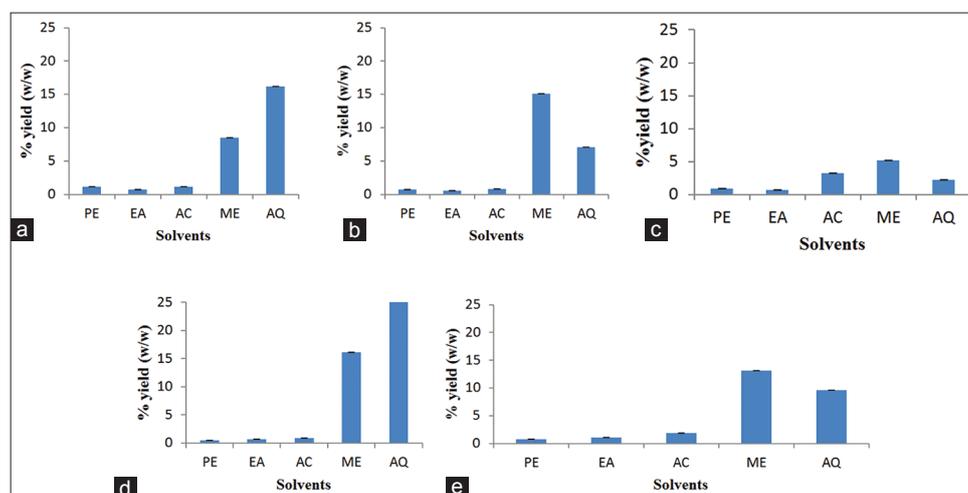


Fig. 1: Extractive yield of different solvent extracts of aerial part of plant (a) *Alysicarpus procumbens*, (b) *Fimbristylis dichotoma*, (c) *Saccharum spontaneum*, (d) *Suaeda nigra*, and (e) *Typha angustifolia*

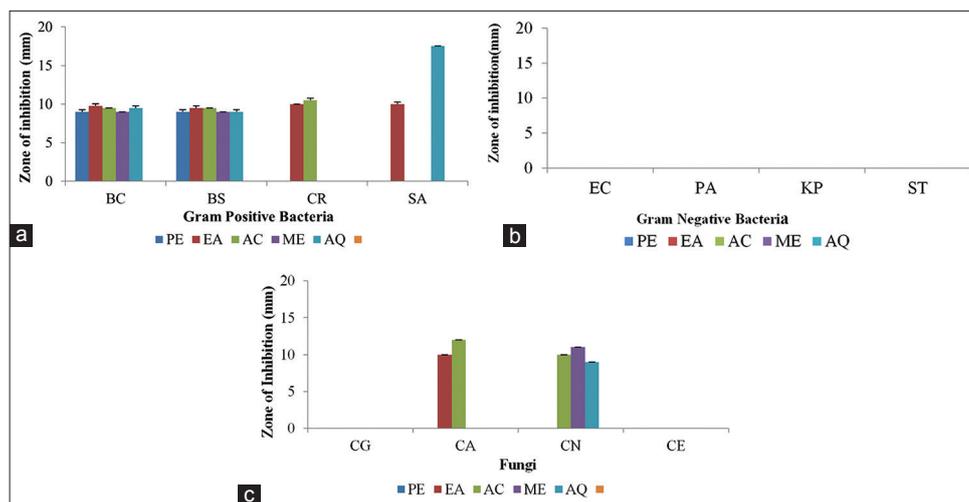


Fig. 2: Antimicrobial activity of different solvent extracts of *A. procumbens* against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi

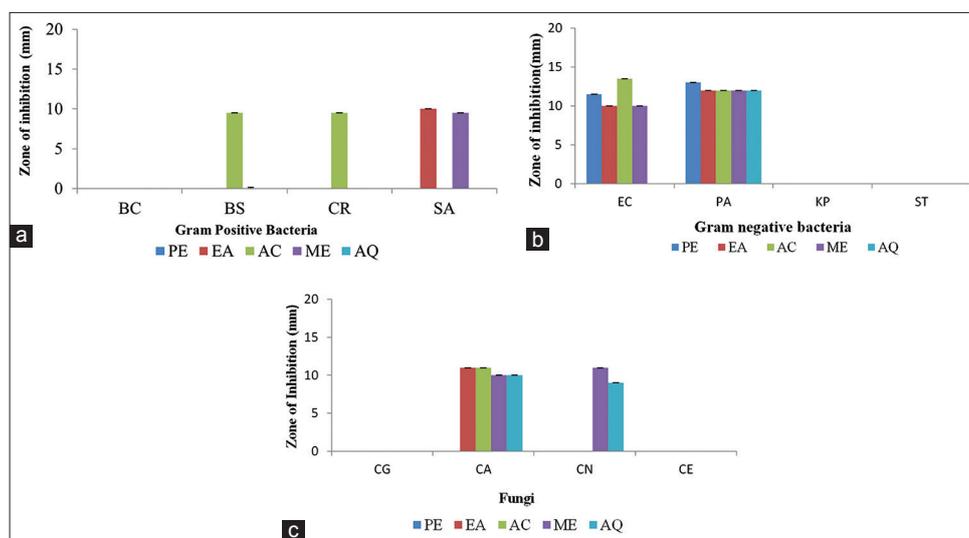


Fig. 3: Antimicrobial activity of different solvent extracts of *F. dichotoma* against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi

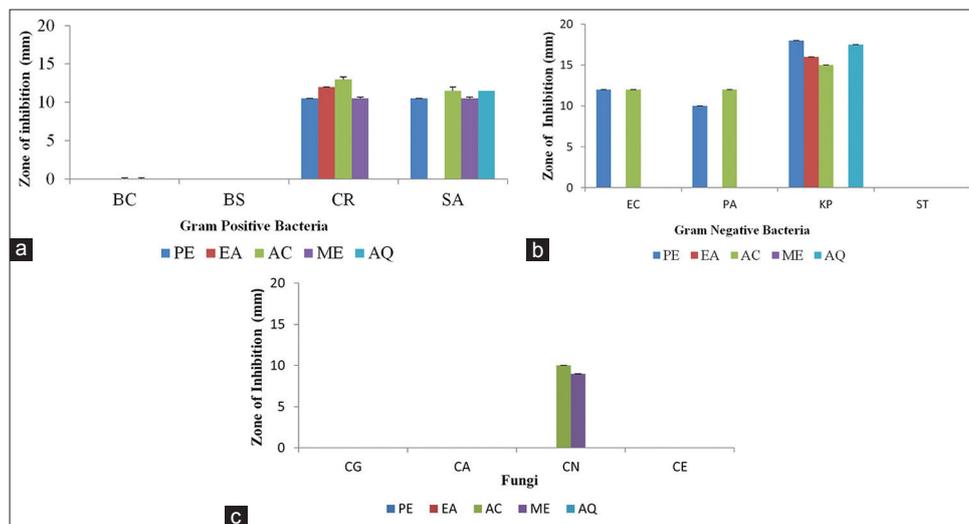


Fig. 4: Antimicrobial activity of different solvent extracts of *S. spontaneum* against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi

extracts except PE extract. *C. neoformans* was inhibited by polar solvent methanol and aqueous extracts (Fig. 3c). In general, methanol and acetone extracts showed maximum antimicrobial activity. *P. aeruginosa* was the most susceptible bacterial strain.

Antimicrobial activity of *S. spontaneum*

Antimicrobial activity of different solvent extracts of *S. spontaneum* aerial part against microorganisms is given in Fig. 4. All the extracts showed good antimicrobial activity toward Gram-positive bacteria and Gram-negative bacteria as compared to fungi. The Gram-positive bacteria, *C. rubrum* and *S. aureus* were inhibited by five extracts expect aqueous and EA, respectively. *B. subtilis* and *B. cereus* were the most resistant bacterial strain (Fig. 4a). The Gram-negative bacteria, *K. pneumoniae*, were inhibited by non-polar solvent PE, semi polar solvent EA, polar solvent acetone, and aqueous extracts. *P. aeruginosa* and *E. coil* were inhibited by non-polar solvent PE and polar solvent acetone extracts. *S. typhimurium* was the most resistant bacterial strain (Fig. 4b). Fungi, *C. epicola*, *C. albicans* and *C. glabrata* were the most resistant fungal strain. *C. neoformans* was inhibited by polar solvent methanol and acetone extracts (Fig. 4c). In general, semi polar solvent PE extract and polar solvent acetone extract showed maximum antimicrobial activity. *C. rubrum* and *S. aureus* were the most susceptible bacterial strains.

Antimicrobial activity of *S. nigra*

Antimicrobial activity of different solvent extracts of *S. nigra* aerial part against microorganisms is given in Fig. 5. All the extracts showed moderate antimicrobial activity against the tested microorganisms. In Gram-positive bacteria, *B. cereus* was inhibited by five extracts expect aqueous extract. *S. aureus* was inhibited by Non Polar solvent PE extracts. *B. subtilis* and *C. rubum* were the most resistant bacteria strain (Fig. 5a). In Gram-negative bacteria, *E. coil* was inhibited by non-polar solvent PE, semi polar solvent EA, and polar solvent acetone extracts. *P. aeruginosa* was inhibited by non-polar solvent PE, semi polar solvent EA, and polar solvent methanol extracts. *K. pneumoniae* and *S. typhimurium* were the most resistant bacterial strains (Fig. 5b). Fungi, *C. glabrata* was inhibited by five extracts expect PE extract. *C. albicans* was inhibited by EA and methanol extracts. *C. neoformans* was inhibited by polar solvent acetone extract. *C. epicola* was the most resistant fungal strain (Fig. 5c). *C. glabrata* and *B. cereus* were the most susceptible bacterial strains.

Antimicrobial activity of *T. angustifolia*

Antimicrobial activity of different solvent extracts of *T. angustifolia* aerial part against microorganism is given in Fig. 6. All the extracts show good antimicrobial activity toward Gram-positive bacteria

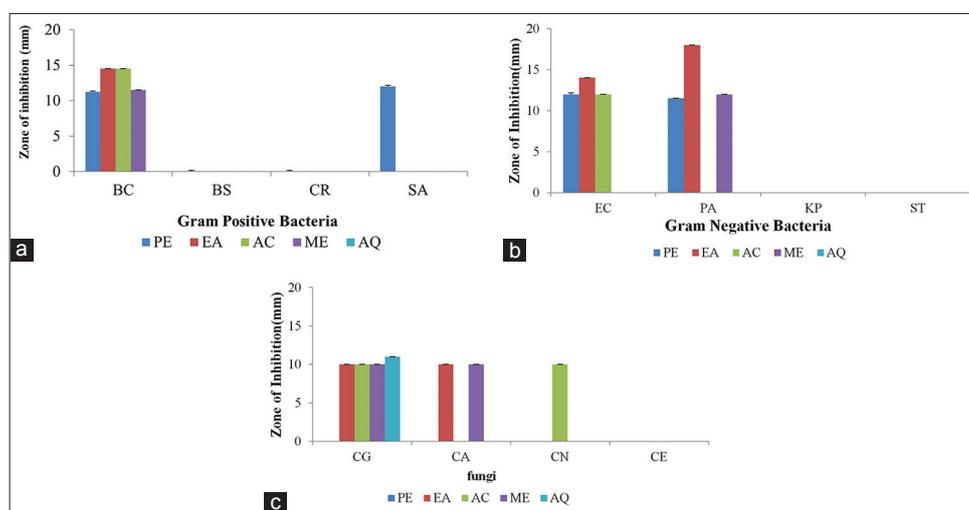


Fig. 5: Antimicrobial activity of different solvent extracts of *Suaeda nigra* against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi

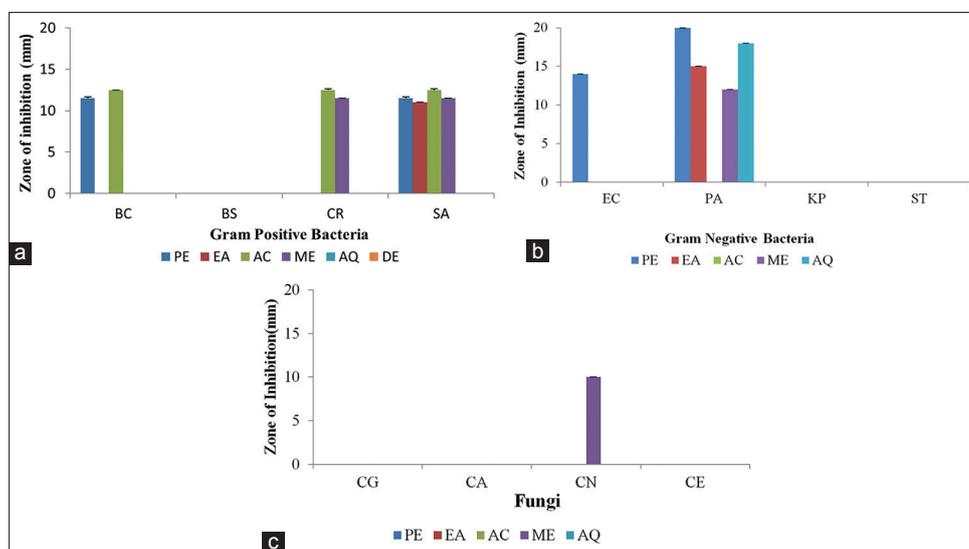


Fig. 6: Antimicrobial activity of different solvent extracts of *T. angustifolia* against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi

and Gram-negative bacteria as compared to fungi. In Gram-positive bacteria, *S. aureus* was inhibited by non-polar solvent PE, semi polar solvent EA, polar solvent acetone, and methanol extracts. *C. rubrum* was inhibited by acetone and methanol extracts. *B. cereus* was inhibited by PE and acetone extracts. *B. subtilis* was the most resistant bacterial strain (Fig. 6a). In Gram-negative bacteria, *P. aeruginosa* was inhibited by five extracts except acetone extract. *E. coil* was inhibited by non-polar solvent PE extract. *K. pneumoniae* and *S. typhimurium* were the most resistant bacterial strain (Fig. 6b). Fungi, *C. glabrata*, *C. albicans*, and *C. epicola* were the most resistant fungal strains. *C. neoformans* was inhibited by polar solvent methanol extract (Fig. 6c). In general, polar solvent methanol extract showed maximum antimicrobial activity. *P. aeruginosa* and *S. aureus* were the most susceptible bacterial strains.

All the five plant solvent extracts showed a different level of antimicrobial activity against different microorganisms. Out of 5 plant extracts, *S. nigra* extracts show broad-spectrum antimicrobial activity. The antimicrobial activity of plant extracts could be due to various phytochemical constituents present in the respective crude extracts [21]. Qualitative phytochemical analysis revealed the presence of a different level of phytochemicals in different plants and they may be responsible for the observed different levels of antimicrobial activity of in all the solvent extracts of five plants [22].

All extracts of five plants showed better antibacterial activity than antifungal activity; Gram-positive bacteria were more susceptible than Gram-negative bacteria. The differences in antimicrobial activity could be attributed to the presences of an additional external membrane surrounding the cell wall in Gram-negative bacteria which blocks the easy penetration of the bioactive compounds [23,24]. Among different extracts of different plants, methanol and acetone extracts displayed better and broad antimicrobial activity; a clear effect of the polarity of the solvents was envisaged. Similar results are reported by Padalia and Chanda [25] in *Tagetes erecta*, Nouri et al., [26] in *Piper betel*, and Morales-Cabrera et al. [27] in *Hibiscus sabdariffa*.

CONCLUSION

All the five plants exhibited a broad spectrum of antimicrobial activity against a wide range of tested microorganism. The solvent extracts of *S. nigra* aerial part exhibited the best activity among the five different plants screened, and thus it can be considered as a good source of the natural antimicrobial agent against infectious disease causing microorganisms. This plant extracts can be further studied for their synergistic approach in treating the multidrug resistant microorganisms.

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